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Administration of *Bacillus subtilis* C-3102 (Calsporin®) may improve feces consistency in dogs with chronic diarrhea

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Abstract

The influence of supplemental *Bacillus subtilis* C-3102 on chronic diarrhea was studied in 40 privately-owned dogs subjected to a double-blind, placebo-controlled trial. After a run-in period of one week, 20 dogs received either placebo or test tablets for four weeks. Test tablets contained 1.3 x 10^8 colony forming units (CFU) *Bacillus subtilis* C-3102 per tablet. Both supplements were administered at a rate of half a tablet per 100 g of air-dry habitual diet, which equals 100 ppm Calsporin in the diet. The owners scored fecal characteristics on a line from 0 to 10 cm. For each dog and each variable, the change over time versus baseline was calculated. In comparison with the control treatment, administration of the probiotic did not influence fecal consistency, color and appearance of blood and mucus. The probiotic significantly improved fecal odor ($P = 0.037$) and tended to reduce flatulence incidence ($P = 0.082$) and apparent fecal fermentation ($P = 0.080$). There was no treatment effect on the degree of tenesmus, failed defecation, fecal volume and coprophagy. The probiotic tended to improve coat condition ($P = 0.058$). Dogs with higher severity degrees of diarrhea during the run-in period showed more improvement during the test period ($P = 0.002$), this relationship being clearly perceptible for the dogs receiving probiotic treatment ($P = 0.056$). It is concluded that administration of *Bacillus subtilis* C-3102 is an effective adjuvant therapy to the treatment of chronic diarrhea in dogs. It is suggested that a well-formulated dog food supplemented with *Bacillus subtilis* C-3102 may optimally support fecal and coat quality.

Keywords:

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Introduction

Probiotics may be described as live microorganisms that, upon ingestion, survive passage through the gastrointestinal tract and exert beneficial effects on the host. Probiotics interact with the host at the levels of the intestinal lumen and epithelium and also beyond the gut. They can contribute to intestinal health by promoting the proliferation of beneficial bacteria, reducing pathogenic pressure through competitive exclusion and stimulating the immune system (Greześkowiak et al., 2015).

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Acute or chronic diarrhea is a common disorder in dogs. The wide variety of pathophysiological mechanisms underlying intestinal disorders with signs of diarrhea makes it difficult to make a proper diagnosis. First-choice therapy for dogs presenting with diarrhea in veterinary practice is directed at the resolution of signs. In a study based on computerized referral histories, it was found that administration of probiotics was used as first-choice therapy in about 10% of the dogs with acute diarrhea (German et al., 2010).

There is evidence that probiotics are effective in the treatment of canine diarrhea. The administration of a Lactobacillus cocktail to dogs with food-responsive diarrhea has been shown to reduce clinical signs (Sauter et al., 2006). In dogs with soft fecal consistency due to food hypersensivity, the feeding of a diet containing *Lactobacillus acidophilus* improved fecal consistency, raised fecal dry matter content and reduced defecation frequency (Pascher et al., 2008). In a placebo-controlled, double-blind study, treatment with a probiotic cocktail shortened the duration of producing abnormal stools in dogs with acute diarrhea (Herstad et al., 2010).

In healthy dogs, the feeding of a diet containing *Bacillus subtilus* C-3102 was found to improve fecal consistency, raise the percentage of fecal dry matter and lowered fecal ammonia concentration (Félix et al., 2010). It was suggested that the probiotic Calsporin®, which consists of *Bacillus subtilis* C-3102, could be effective in the treatment of diarrhea in dogs. This suggestion was put to the test in a double-blind, placebo-controlled trial in which privately-owned dogs with chronic diarrhea received tablets containing *Bacillus subtilis* C-3102.

Materials and Methods

Dogs with chronic diarrhea were recruited through mounting posters in petshops, supermarkets and pet pensions. In addition, an email with details of the projected trial was sent to 1000 dog breeders, 220 veterinarians and 200 breed and dog-fancier clubs. On various dog websites and in magazines, details of the trial and a call-up for canine participants were placed. Owners put their dogs forward by sending an email to J.H. Van der Laak and J.I. Smit or by contacting them by telephone.

Upon presenting a canine participant, a questionnaire was sent to the owner. Through the questionnaire, information was obtained about characteristics of the dogs offered and the amount and type of food fed. Exclusion criteria were exocrine pancreatic insufficiency and lack of diarrhea. One dog was excluded just before entering because of required antibiotic treatment of an intestinal infection. Forty dogs from various breeds were enrolled. There were 23 males and 17 females aged between 12 months to 13 years. One dog received Dexoral® as pharmaceutical because of atopy, and another dog was given Bach blossom as supplement.

Owners with elected dogs received the trial booklet and the control or test tablets by surface mail. The degree of feces consistency as indicated in the questionnaire was used by A.C. Beynen to enter animals into either the control or test group so that the group distributions of fecal consistency would be similar. For the other authors and the owners, the treatment of each dog was masked until data analysis and reporting the outcome. The trial lasted five weeks during which fecal characteristics were graded in the trial booklet. The first week served to determine baseline values. During the second to fifth week, control or test tablets were administered. The owners were instructed to continue feeding their dog's habitual diet during the whole trial period.

Three quarter of the dogs received diets that consisted of commercial kibbles. One quarter of the dogs received a BARF (Bones And Raw Food)-compatible homemade ration alone or in combination with commercial frozen food. The tablets were specially produced for the trial. They weighed 0.5 g each and consisted of brewers' yeast (*Saccharomyces cerevisiae*), aroma and colloidal silica. The control and test tablets were undistinguishable. In the test tablets, the CFU of *Bacillus subtilis* C-3102 (Calsporin®) was found to be 1.3 x 10^8 CFU per tablet.

The daily dose of probiotic was related to each dog's intake of dietary dry matter and so to its approximate energy intake. In this way, the dose was standardized among dogs with different body weights. For each 100 g of kibble, a dog received one half control or test tablet. The 100 g kibble equivalents for frozen and canned food were taken to be 230 and 500 g, respectively. Each owner was instructed to administer to its dog(s) a number of tablets to the nearest 100 g of kibble or the nearest equivalent as fresh, frozen or canned food.

In the booklet, each owner registered scores for the various observations. The following variables were scored by the owners: condition of coat, vomiting, body condition, activity, fecal characteristics (color, consistency, odor, amount and presence of blood, mucus and coarse constituents) and defecation characteristics (frequency, tenesmus, failed defecations, coprophagy). The booklet described the symptoms and their range of qualities. For scoring, a vertical 10 cm line was used with a description of the variable concerned in terms of highly aberrant towards ideal. Fecal and defecation characteristics were scored daily and the other variables weekly.

After completion of the trial, the booklets were collected and the marks on the lines were measured in mm so that scores reflected a 0-100 scale. Ideal scores were 100, 60 (fecal consistency) and 50 (body condition, fecal color, amount of feces). Scores of variables with 100 as ideal score were used as measured. Other score values were adjusted by comparing to the ideal score of 50 (color, amount) or 60 (consistency). The average scores for the first week were used as baseline values and those for week 5 as final values. For each dog and each variable the difference between final and baseline value was calculated.

To identify treatment effects, the changes over time for the placebo and test groups were subjected to the Student's t-test with P<0.05 as criterion of statistical significance. Paired sample t-test was used to measure the difference within groups. Generalized linear models were used to analyze the effect of the initial score (baseline value) on the change over time. Statistical analyses were performed using SPSS statistic 20 and Genstat 16th edition.

Results

The 40 dogs enrolled completed the trial and the data are based on 20 dogs in each treatment group. The general characteristics of the control and test dogs were similar. Their mean age was 55.9 and 52.0 months, body weight was 27.9 and 28.8 kg, and height at withers was 55.9 and 56.2 cm for the control and probiotic group respectively.

Group mean baseline values and changes over time for the fecal characteristics and variables related to general health are presented in Tables 1 and 2, respectively. Improvement is indicated with + sign and deterioration with – sign. The scale ran from 0 "often and/ or severe clinical signs" to 100 "no signs".

The changes over time for the following fecal characteristics were not influenced by the test versus control treatment: consistency, color, blood and mucus appearance (Table 1). Fecal odor was significantly improved (P = 0.037) and the degree of apparent fermentation tended to be reduced by the probiotic (P = 0.080). There were no group differences in the degree of tenesmus or failed attempts of defecation. Likewise, there was no treatment effect on fecal volume and coprophagy. Probiotic treatment tended to diminish flatulence incidence (P = 0.082). In quantitative terms, the beneficial effects on fecal odor, fermentation and flatulence as mediated by *Bacillus subtilis* C-3102 were in the order of 5 to 10 on the 0 to 100 scale. Table 2 documents that coat condition tended to be improved by probiotic treatment (P = 0.061).

On the score line of fecal consistency, the value of 0 represents watery diarrhea and that of 100 very hard feces. Optimum fecal consistency would obtain a score of 60 so that any changes in the degree of diarrhea are in the 0 to 60 range. In the first week, 15 control and 16 test animals had fecal consistency scores below 60, the group means being 41.8 and 43.0. The group-mean changes over time were similar in the two groups (Table 1).

Figure 1 shows relationship between the individual fecal consistency scores in week 1 (baseline value) and the improvement during the treatment period for control and test dogs. Regression analysis detected a tendency toward interaction between both the X and Y values (P = 0.071). Dogs with low scores for fecal consistency before treatment showed more improvement over time during the experimental period (P = 0.002), the relationship being clearly perceptible in the probiotic-treated dogs (P=0.056). The regression line predicts that the probiotic upgrades a pre-treatment value of 20 to a post-treatment value of 57.

Table 1: Baseline values and changes over time in the fecal characteristics (improvement is indicated with a + sign)

Variable	Control (n=20)				Probiotic (n=20)				P value for between group difference in change (2-tailed)
	Baseline	Change vs baseline	P value for change (2-tailed)	SEM	Baseline	Change vs baseline	P value for change (2-tailed)	SEM	
Consistency*	41.8	+ 10.3	0.018	3.87	43.0	+12.6	0.003	3.54	0.667
Odor	67.2	- 5.8	0.112	3.49	45.1	+10.2	0.131	6.47	0.037
Color**	-12.6	-3.2	0.139	2.08	-12.3	+0,8	0.645	1.62	0.141
Blood	99.1	-0.6	0.303	0.53	99.8	0.0	0.900	0.06	0.311
Mucus	93.9	+2.8	0.236	2.28	92.8	+5.9	0.031	2.53	0.370
Fermentation	97.3	-4.4	0.158	2.97	91.4	+2.4	0.311	2.30	0.080
Tenesmus	89.8	+2.7	0.277	2.37	85.7	+3.0	0.310	2.95	0.911
Failed defecation	93.8	+1.4	0.388	1.52	94.4	+4.6	0.130	2.93	0.328
Flatulence	88.2	-5.1	0.015	1.91	94.1	+1.0	0.723	2.86	0.082
Amount **	-8.1	-1.7	0.361	1.79	-10.3	-4.7	0.226	3.57	0.490
Coprophagy	73.5	+4.7	0.102	2.74	77.7	+8.3	0.053	4.02	0.462

*Selection of dogs with baseline score 0 (diarrhea) to 60 (ideal). Dogs with dry feces (baseline score >60) are excluded from the score on consistency. Control treatment: n=15; probiotic treatment: n=16; ** Score change compared to ideal score of 50.

Table 2: Baseline values and changes over time for variables related to general health (improvement is indicated with + sign)

Variable	Control (n=20)				Probiotic (n=20)				P value for between group difference in change (2-tailed)
	Baseline	Change vs baseline	P value for change (2-tailed)	SEM	Baseline	Change vs baseline	P value for change (2-tailed)	SEM	
Coat condition	89.4	-3.9	0.076	2.05	87.0	+5.3	0.224	4.18	0.061
Vomit	90.7	+1.7	0.620	3.37	92.9	+1.9	0.212	1.47	0.957
Body condition*	43.2	+1.0	0.602	1.89	35.8	+4.0	0.071	2.07	0.299
Activity	83.7	+1.1	0.560	1.86	81.2	-4.1	0.445	5.25	0.360

*Scale 1-100, with 1 = very skinny, 50 = good condition and 100 = obese.

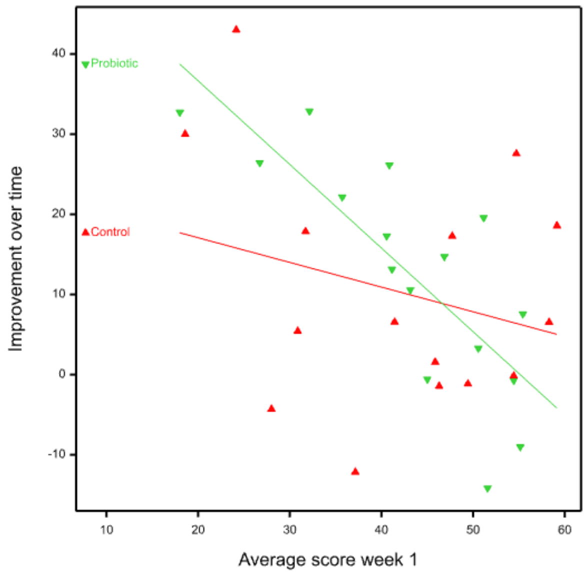

Fig. 1: Relationship between pre-treatment fecal scores ranging from 0 to 60 (0 = diarrhea; 60 = ideal) and fecal consistency improvement during the treatment period in individual control dogs (n = 15) and probiotic-supplemented dogs (n=16). For control and test dogs the linear correlation coefficients were 0.26 and 0.79, respectively.

Discussion

This study shows that administration of *Bacillus subtilis* C-3102 improved fecal consistency more clearly in dogs with more severe chronic diarrhea. On a group basis, the probiotic significantly improved fecal odor and reduced mean flatulence incidence and apparent fecal fermentation. The present observations corroborate earlier studies demonstrating the beneficial impact of Lactobacillus-containing probiotic preparations on diarrhea in dogs (Sauter et al., 2006; Pascher et al., 2008; Herstad et al., 2010).

The mechanism of action of probiotics in the treatment of canine diarrhea may relate to changing the intestinal microbiota. Oral administration of *Lactobacillus acidophilus* strain DSM13241 to healthy dogs lowered both the number and the relative percentage of fecal clostridia (Baillon et al., 2004). When healthy dogs were given *Enterococcus faecium* NCIB 10415, total counts of clostridia in feces were suppressed, but those of salmonella and campylobacter were unchanged (Vahjen and Männer, 2003). It is likely that probiotics counteract the colonization of clostridia in dogs and thus may protect them from clostridial infection and disease.

Two independent studies have indicated that ingestion of probiotics stimulates the immune system in healthy dogs. The intake of *Enterococcus faecium* SF68 raised the concentration of total immunoglobulin (IgA) in feces and also the levels of specific anti-canine distemper virus IgG and IgA in blood plasma (Benyacoub et al., 2003). Oral administration to healthy dogs of *Lactobacillus acidophilus* strain DSM 13241 was found to increase the concentrations of neutrophils, monocytes and IgG and to reduce the concentration of nitric oxide in blood (Baillon et al., 2004). The observed immunological effects of the two probiotic preparations can be considered advantageous and could contribute to recovery from diarrhea.

An interesting finding that emerged from this study is that *Bacillus subtilis* C-3102 improved coat condition, which nearly reached statistical significance. A similar observation has been made in arctic foxes: the administration of a mixture of *Enterococcus faecium* and *Lactobacillus acidophilus* from weaning improved pelt quality (Gugolek et al., 2004). Thus, the positive effect of *Bacillus subtilis* C-3102 on coat condition may not be spurious.

Many dog owners assess their dogs' feces and coat. They regard fecal feces quality as an indicator of intestinal health and food digestibility. A strong, shiny hair coat is seen as a sign of good general health. Both fecal and coat condition are considered to reflect the quality of the food being fed. This study shows beneficial effects of *Bacillus subtilis* C-3102 on fecal and coat condition in dogs with chronic diarrhea, which may extend to a supportive and preventive function of the probiotic. This implies that a well-formulated dog food containing a probiotic such as *Bacillus subtilis* C-3102 would be highly appreciated by dog owners.

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